ORAL PATHOLOGICAL DIAGNOSTIC

#529

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Application For Research Grant

Date: 31 January 1966

1. Name of Investigator: John H. Manhold, Jr., D.M.D., M.A., F.A.C.D., F.I.C.D.

2. Title: Professor and Director of Pathology and Oral Diagnosis for the College of Dentistry

3. Institution & Address: New Jersey College of Medicine and Dentistry
Jersey City, New Jersey 07304

4. Project or Subject: To study the purported relationship between smoking and changes in human oral tissue in vivo by routine microscopy, differential staining, and micro-respirometer methods, and to further examine statistically two series of data presently in the principal investigator's possession.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

Introductory data -- The relationship between smoking and malignant or premalignant changes in bronchial and lung tissue has been much studied. Additionally, oesophageal changes with smoking have been studied at some length by, among others Wynder and Bross[1] and Auerbach[2] (who incidently also is one of the investigators who has published several articles on bronchial and lung changes associated with smoking).[3-7] The oral cavity per se has not been as well studied although many statistics and much conjecture exist in regard to a purported relationship between smoking and the occurrence of malignancy in this area. However, the statistics stem largely from linear correlations which continue to offer considerable controversy and the conjecture, along with some of the statistics, stem from animal experimentation done with the use of "smoke boxes" and/or the application of various carcinogenic agents. Actual human in vivo experimentation from other than the statistical viewpoint, is not extensive. Kirsch,[8] for example, has suggested the existance of a difference in salivary pH between smokers and non-smokers. Pindborg,[9] from the results of microscopic study of a small sample, has suggested that cigarette smoking produces a hyperparakeratotic condition in the oral cavity. Wrubel and Scopp,[10] using oral exfoliative cytologic methods have reported no change in the hard palate and buccal mucosa with cessation of smoking. On the other hand, Zimmerman and Zimmerman,[11] using the same method, have reported significant differences in oral tissue keratinization between smokers and non-smokers.

A certain lack of confidence exists among many pathologists in regard to oral exfoliative cytologic techniques. Peters[12] has demonstrated some basis for this lack of confidence, and Manhold et al,[13] have demonstrated a further apparent unreliability of the oral cytologic technique by not being able to find histological evidence to corrobrate the cytologic results reported by Derbyshire and Manhodi.[14]

Nevertheless, these various studies by Kirsch, Pindborg, Wrubel and Scopp and Zimmerman and Zimmerman, when considered in relation to studies done by Cahn and associates,[15] suggest the possibility of more exact human in vivo investigation of the oral tissue-smoking problem. For example, Cahn, Eisenberg and Blake suggested in 1961 that it may be possible to distinguish potentially malignant epithelium from benign epithelium by study of the basement membrane with Periodic Acid Schiff[15] Stain and in 1962 these same authors suggested that the existance of parakeratosis without glycogen may be "a sinister sign in the prognosis of white lesions of the mouth."[16] They also suggested in this same study that a difference in RNA could be seen with methyl-green pyronine.

It is proposed, therefore, to study the purported relationship between smoking and changes in human oral tissue in vivo by routine microscopy, differential staining, and microrespirometer methods, and to further examine statistically two series of data presently in the principal investigator's possession in the following manner:

PROCEDURE

Part I

It is proposed that a group of individuals who are heavy cigarette smokers and a group who are non-smokers have an area biopsied from the oral mucosa and that this biopsy be submitted to histologic study with H & E, Periodic Acid Schiff, Methyl-green Pyronine, and Feulgen Stain methods. Simultaneously part of these tissues will be subjected to tissue microrespirometry to ascertain whether any oxygen consumption difference appears to exist between the tissue of the smokers and non-smokers.

The staining techniques employed will be standard and will be utilized to compare with the results presented by Pindborg, Wrubel and Scopp, Zimmerman and Zimmerman and Cahn et al. The microrespirometer technique to be utilized here is fundamentally that initiated by Kirk.[18] It will employ the Stern-Kirk microrespirometer in the manner described by Manhold and associates,[19-26] and will allow comparisons with the values reported by these authors for normal and inflammed tissues.

Part II

In addition to this procedure the following is suggested:

Over the past seven years the proposed principal investigator has conducted a Biopsy Service for dentists of the State of New Jersey and environs. This service now processes in excess of 1,000 specimens per year. Over the past year and one-half there have been, among the biopsies submitted, 37 patients whom we have diagnosed as having lesions of a fundamentally hyperkeratotic nature; 8 patients who have been diagnosed as having invasive carcinoma; and there have been 2 patients who have been diagnosed as having leukoplakia. I propose first to contact the surgeons who submitted these specimens so as to gain pertinent data in regard to smoking habits, use of alcohol, and any medical or oral history which could be pertinent.

With these data for comparison, sections from blocks of these lesions will be cut and re-examined microscopically with appropriate H & E and differential staining as described above in Part 1. (If any significant trend appears from this preliminary perusal, additional information could later be gained by tracing all cases of this type back through the remaining six years of our Biopsy Service. This work could also then be expanded to study any epithelial hyperplastic change. Further work in this area would, as stated, depend upon the apparent fruitfulness of this preliminary survey and would however, constitute another area of endeavor, which would necessitate further planning and additional funds.)

Part III

Because production of oral carcinoma is thought by many to stem largely from irritation, and because changes in the normal physiology of components of the oral cavity can be thought to be part of this irritational factor and also because Kirsch[8]

has suggested that a difference exists in the pH of the saliva of smokers and non-smokers, it might well prove interesting to ascertain whether this reported difference can be replicated in another sample. We have already accumulated data on a number of individuals and established a relationship between saliva, dental calculus formation and Blood Serum Phosphorus.[27] Thus we have pH and quantity of saliva on this group. We have, additionally but as yet unanalized, data on these individuals as to whether or not they are smokers. To examine these relationships will require merely a T-test or critical ratio on these data.

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JOHN H. MANHOLD, JR., B.A., D.M.D., M.A., F.A.C.D., F.I.C.D.

REDACTED

2. Professional History in Brief:

REDACTED

3. Education:
B.A., University of Rochester 1940
D.M.D., Harvard University 1944
Fellow in Pathology, University of Rochester 1944
Certificate in Medical Aspects of Atomic, Bacteriological, Chemical Warfare: Army Chemical Corps School, Maryland 1950
M.A., in Psychology, Washington University 1956

4. Honors:
International College of Dentists (Fellow)
American College of Dentists (Fellow)
Society of the Sigma Xi (Member)
Omicron Kappa Upsilon (Member)

5. Biography in Who's Who in the East (since 1950), American Men of Science (Vol. II), and Leaders in American Science.

6.

b.

REDACTED

c.

7. Positions (from present to earliest)
Professor and Director of Pathology and Oral Diagnosis, Seton Hall (now New Jersey) College of Medicine and Dentistry, 1957-; Secretary to the Executive Faculty, 1957-; Coordinator of Cancer Teaching, 1956-; Attending in Pathology, Jersey City Medical Center 1958-; Coordinator of Research 1956-1960.
Associate Professor and Director of Pathology, College of Dentistry, Seton Hall College of Medicine and Dentistry, 1957-1957.
Assistant Professor and Director of Pathology, College of Dentistry, Seton Hall College of Medicine and Dentistry, 1956-1957 (Interim Appointment).

Assistant Professor and Acting Head of the Departments of General and Oral Pathology, Washington University School of Dentistry 1954-1956.

Research Officer and for several months Senior Research Officer Present, Aviation Psychology Laboratory and Research Pathologist, Naval School of Aviation Medicine, N.A.S., Pensacola, Florida 1952-1954.

Assistant Force Dental Officer, Fleet Marine Forces of the Pacific, 1951-1952.

Assistant Research Officer and Lecturer on Medical Aspects of Atomic Explosion, U.S.N.T.C., Great Lakes, Ill., 1950-1951.

Director, Cancer Teaching Program and Instructor, Oral Pathology, Tufts University College of Dentistry 1948-1950.

Private practice for a short time after World War II in which service was performed in the Navy for two years.

Designated by the Bureau of Medicine and Surgery of the United States Navy as "Qualified for Independent Research Duty".

Formerly Principle Investigator of U.S. Navy Research Project NM 001 057.11

8. Publications:

A. Books

1) INTRODUCTORY PSYCHOSOMATIC DENTISTRY by Manhold, J. H., Jr. Appleton-Century-Crofts, New York, 1956.
2) OUTLINE OF PATHOLOGY by Manhold, J. H., and Bolden, T. E. W. B. Saunders Co., Philadelphia, 1960.
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6) CLINICAL ORAL DIAGNOSIS by Manhold, J. H., Jr., McGraw-Hill, New York, 1965.

b. Papers (Abstracts are not included unless subsequently published as complete papers on the subject presenting substantially additional material.)

Early Research Reports:

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2) Glickman, I., Turesky, S., and Manhold, J.: The Oxygen Consumption of Healing Gingiva. Jour. Dent. Research 29: 429, 1950.

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Papers since affiliating with Seton Hall:

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PRELIMINARY REPORT OF RESPIRATION CHANGES IN RAT GLAND TISSUE AS A RESULT OF LIGATION. J.H. Manhold, Jr., T. E. Bolden, and M. L. Hall. The exorbital lacrimal gland of the rat exhibits many similarities to the salivary gland, and is more advantageous for study. A normal respiratory quotient (QO_2 = 0.41± 0.14) was established for exorbital lacrimal gland tissue of 150 day old male Wistar rats by use of a microrespirometer technique (Manhold, Bolden, and Katz 1959). Excretory ducts were then ligated, and the animals killed at 1, 2, 3, 7, 14 days, 1 month, and 4 months post-ligation. The duct-ligated glands were removed, placed in Tyrode's solution, and divided into two parts. Half was fixed for microscopic study. Half was placed in microrespirometers for QO_2 determination. Results were: 1 day - (11 glands) 0.36± 0.59; 2 days - (9 glands) 0.34± 0.22; 3 days - (8 glands) 0.70± 0.08; 7 days - (12 glands) 0.64± 0.53; 14 days - (11 glands) 0.50± 0.14; 1 month - (10 glands) 0.29± 0.25; 4 months - (10 glands) 0.30± 0.10. These results corresponded with the microscopic picture. Tentatively, therefore, it may be concluded that duct-ligation caused an initial drop in the respiration of glandular tissue followed on the third day by an increase. A constant QO_2 value, which is lower than the initial value, is reached when the gland histologically shows more scar tissue and less parenchyma.

Presented at the 39th General Meeting of the International Association for Dental Research, Boston, Massachusetts, March 1961.

COMPARATIVE STUDY OF SUBMAXILLARY AND LACRIMAL GLANDS OF THE RAT. Theodore E. Bolden and John H. Manhold, Jr. Striking similarities in composition and response to disease processes have been demonstrated for salivary and lacrimal glands. This study determined the composition and dry weight of lacrimal glands and compared some of the data with data obtained in a similar fashion for submaxillary glands. Lacrimal glands from seven groups of untreated male Albino rats were used. (15, 30, 60, 90, 150, 300 and 500 days.) A quantitative estimate was made of the relative areas in sections of left glands which were occupied by acinar and by intralobular duct tissue. An ocular grid divided into 66 squares was used. A total of 1200 squares were counted. The number of squares in which half or more of the area was occupied by acini or ducts was counted in 3 specimen from each age group. Dry weight determinations were made on the right gland of 27 rats from the last six ages. The relative proportion of acinar tissue to intralobular duct tissue remained fairly constant at all ages. The normal gland consisted of about 82% acinar tissue, and 3% intralobular duct tissue. Lacrimal glands consisted of approximately 24% more acinar tissue and 22% less intralobular duct tissue than the submaxillary glands from rats of the same age. An average dry weight of 8 mlg. (30 days) was increased to 50 mlg. (60 days) fell, and fluctuated around 25 mlg. at all other ages. The ratio of gland weight to body weight was 0.75 and .189 respectfully, for lacrimal and submaxillary glands.

Presented at the 39th General Meeting of the International Association for Dental Research, Boston, Massachusetts, March 1961.

MICRORESPIROMETER TECHNIC FOR STUDY OF HUMAN GINGIVAL TISSUE: PRELIMINARY REPORT.

J. H. Manhold, Jr., T. E. Bolden, and S. Katz,
Seton Hall College of Medicine and Dentistry,
Jersey City.

Glickman, Turesky, and Hill (1949) employed the Warburg manometric technic to study human gingival metabolism. Because accurate Warburg determinations require relatively large quantities of tissue (40-50 mg. wet weight) and such quantities of human tissue usually are not readily available, Glickman, Turesky, and Manhold (1950) employed dogs to study oxygen requirements of healing gingival tissue. Exact relationships between human and dog gingiva Q_{O_2} values were not found. Further investigations did not follow. Kirk (1950) presented a quantitative ultramicroanalysis method for measuring the metabolism of tissue cultures. This technic, giving accuracy on 10 mg. or less (wet weight) of tissue, appeared ideal for human gingival study and was used in the present investigation. Small sections of gingival tissue, removed from clinic patients of Seton Hall College of Medicine and Dentistry, were placed in Tyrode's solution and divided into two approximately equal parts. Half was fixed for microscopic study. Half was placed in microrespirometers for Q_{O_2} determinations. Thirty-six samples of microscopically determined normal and essentially normal gingival tissue yielded a mean Q_{O_2} of 1.39 ± 0.46, a value comparable to earlier results. Microrespirometer investigation of human gingival tissue appears feasible, therefore, and presents the advantage of using small tissue quantities.

J. D. Res., July-August 1960, Vol. 39, #4, pg. 746-7, #265.